Structural Examination of the Nickel Site in *Chromatium vinosum* Hydrogenase: Redox State Oscillations and Structural Changes Accompanying Reductive Activation and CO Binding[1]

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Introduction: Hydrogenases (H_2ases) are enzymes that catalyze the two-electron redox chemistry of H_2. The H_2ase isolated from *Chromatium vinosum*, a purple-sulfur, photosynthetic bacterium, is a typical member of the class of H_2ases having a [NiFe] dinuclear active site. The studies reported here examine the structure of the Ni site in eight states of the enzyme related by redox poise, one state that is a photoproduct of Form C, the reduced, epr active state of the enzyme, and a CO inhibitor complex. These states and their relationships are summarized in Figure 1. The studies were specifically designed to examine the presence/absence of a Ni-O ligand as a function of redox poise, to examine the structure of the CO complex and determine the metal binding site, and examine systematically the changes in the Ni-Fe distance as a function of redox level.

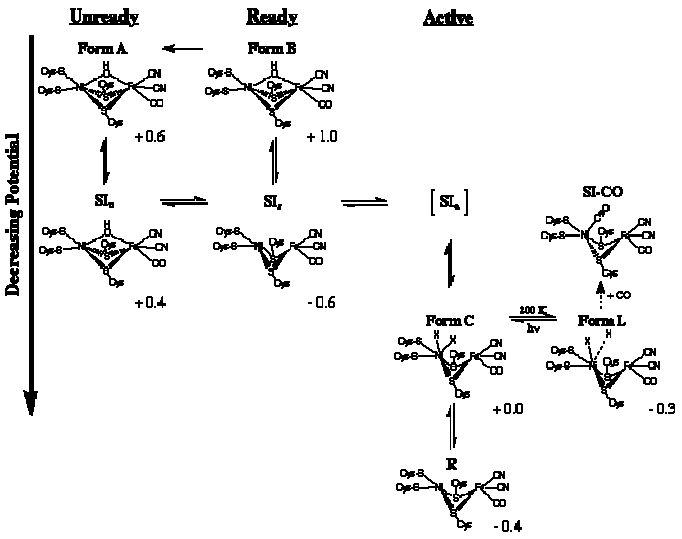

Figure 1. A summary of the chemistry of the active site of *Chromatium vinosum* H_2ase. The structures shown are consistent with EXAFS and XANES analyses. The numbers indicate the shift in the Ni K-edge energy from the average value of the samples, 8339.4 (1) eV.

Methods and Materials: Ni K-edge XAS was performed on beamline X9B using frozen samples held near 50K in a displex unit. X-ray fluorescence data was collected using a 13-element Canberra detector. Data analysis was carried out using the program Jinxes. The redox poise of samples was determined by EPR for EPR active states on the XAS samples without thawing them. The redox poise of EPR-silent states was determined by IR before freezing the samples. The integrity of the samples upon exposure was determined by a combination of three methods. First the Ni K-edge of the samples was monitored on sequential scans to determine if the redox poise of the sample was changing. Second, the redox poise of the samples was verified after exposure using by spectroscopy. Third, the activity of the samples was measured before and after exposure. There was no evidence for radiation damage to the samples.

Results and Conclusions: An x-ray absorption spectroscopic study of structural changes occurring at the Ni site of *Chromatium vinosum* hydrogenase during reductive activation, CO binding, and photolysis was completed. Structural details of the Ni sites for the ready silent intermediate state, SI_r, and the carbon monoxide complex, SI-CO, were delineated for the first time in any hydrogenase. Analysis of nickel K-edge energy shifts in redox-related samples reveal that reductive activation is accompanied by an oscillation in the electron density of the Ni site involving formally Ni(III) and Ni(II), where all the epr active states (Forms A, B, and C) are formally Ni(III) and the epr silent states are formally Ni(II) (Figure 1). Analysis of XANES shows that the Ni site undergoes changes in the coordination number and geometry that are consistent with five-coordinate Ni sites in Forms A, B, and SI_u, distorted four-coordinate sites in SI_r and R, and a six-coordinate Ni site in Form C. EXAFS analysis reveals that the loss of a short Ni-O bond accounts for the change in coordination number from five to four that accompanies formation of SI_r. A shortening of the Ni-Fe distance from 2.85(5) Å in Form B to 2.60(5) Å also occurs at the SI level and is thus associated with the loss of the bridging O-donor ligand in the active site. Multiple-scattering analysis of the EXAFS data for the SI-CO complex reveals the presence of Ni-CO ligation, where the CO is bound in a linear fashion appropriate for a terminal ligand. The putative role of Form C in binding H_2 or H^- was examined by comparing the XAS data from Form C with that of its photoproduct, Form L. The data rule out the suggestion that the increase in charge density on the NiFe active site that accompanies the photoprocess results in a two-electron reduction of the Ni site (Ni(III) -> Ni(I))[2]; only subtle structural differences between the Ni sites were observed

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References: [1] G. Davidson *et al.*, *Biochemistry*, **39,** 7468-7479 (2000). [2] R. Happe, W. Roseboom, S. Albracht, *Eur. J. Biochem.* **259**, 602- 608 (1999).